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Improved stability and spectral quality in ex situ dissolution DNP using an improved transfer device

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ABSTRACT

Dissolution DNP has become one of the predominant implementations for dynamic nuclear polarization. However, the technical implementation of transferring the sample from the polarizer to the NMR system remains challenging. There is a need for additional technical optimizations in order to use dissolution DNP for biochemical and chemical applications. Here we show how a newly designed pressure dissolution kit considerably improves spectral quality and stability by enabling highly reliable and fast sample transfer to the NMR system.

1. Introduction

Dissolution DNP has been quickly established as a research tool to study DNP, and has great potential as an add-on for a series of NMR applications. The concept of polarizing at low temperatures (typically 1-3-1.4K) [1] yields the highest possible polarizations by combing the actual polarization with a temperature factor (298K/1.4K~200). However, the concept has serious inherent shortcomings, mostly arising from the necessity to melt the sample in a very short time, and to transfer it into an NMR tube in a different magnet for the NMR measurements. In the process the sample forms bubbles, and the transfer is suboptimal.
Hilty and coworkers [2] introduced a system that keeps the dissolved sample under pressure after the dissolution, thus avoiding bubbles in the sample in the NMR magnet. A pressure gradient helps to speed up the transfer, and measurements can start immediately, as the sample doesn't need to settle and degas after the transfer. The implementation of this dissolution system has been challenging as sample tubes tend to break under the sudden pressure change, and the implementation using Labview is relatively expensive.
We have therefore redesigned this dissolution system for a simpler and mechanically more stable design. A robust and flexible microprocessor based design has been developed that can readily be adapted to other polarizers. A new optical flow detector allows for reliable triggering of the transfer process. A graphical interface facilitates the use of this post-dissolution device. Here we describe the newly designed concept along with applications that demonstrate a considerable improvement.
The setup employs the open-source Arduino architecture along with a customized sample holder. This architecture is sufficiently flexible to be adapted for other uses, including controlled release of a polarized sample onto cells for metabolic flux experiment. The system has been tested using a Hypersense polarizer, but should be applicable to any comparable system. Our data shows considerably improved quality of one and two-dimensional spectra and substantially improved data quality for longitudinal relaxation measurements.

2. Implementation

We used an Oxford Instruments Hypersense polarizer along a Bruker Avance500 III NMR spectrometer, equipped with a broadband 5mm probe. The dissolution device was built to minimize the sample transfer time and to optimize sample stabilization in conjunction with this equipment. It triggers the spectrometer after a user adjustable time period.

The overall arrangement of the dissolution device is shown in Fig. 1 (Fig.1 S1 for the injection position). A high-pressure manifold (Fig. S3) splits the input pressure into two independent output pressures, one for pre-pressurizing the NMR tube (p_{LP}), the second to transfer the sample into the NMR tube and to keep it pressurized for the NMR measurement (p_{HP}). An electronic valve assembly consisting of 4 SMC VDW31 valves switched by 4 channel 5V multi-relay module from Sainsmart opens the outputs of the manifold to the pressure lines.

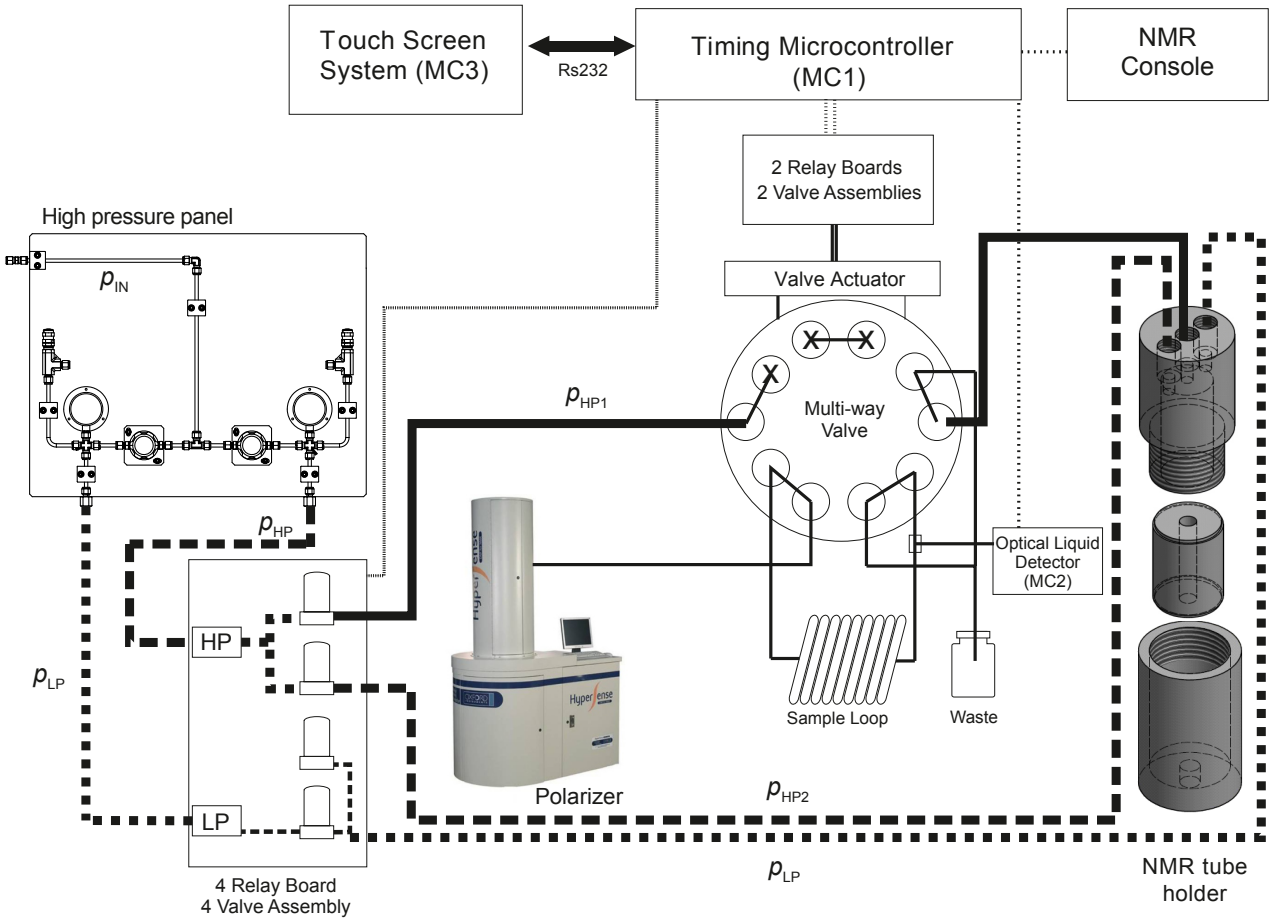

Fig. 1: Overall design of the pressure dissolution system showing the individual components and the connections in the system. The microcontrollers (MC) are used to control the pneumatic assembly. The high pressure panel splits the input pressure into a low output pressure p_{LP} for prepressurizing the device and a high input pressure p_{HP} to transfer the sample to the NMR system and to keep it under pressure after the transfer.

Overall operation procedure. After dissolution of the sample at 1.3K by pressurized solvent in the Hypersense it is transferred into the sample loop of a multi-way two-position valve (VICI C22 6180, 2 position 10 I/O valve with pneumatic actuator) with a pneumatic valve actuator (VIVI valco).

An optical detector triggers the switch of the multi-way valve to inject the sample under a pressure of p_{HP} into the NMR tube (Fig. S4). While line HP1 is constantly kept at the high pressure of p_{HP}, line HP2 is pressurized with a delay of typically 600ms –the time of the sample transfer– to stabilize the sample in the NMR tube after transfer. The acquisition of the NMR spectrum is automatically triggered with a delay of typically 1-2s after switching the multi-way valve. With this

the overall transfer until the measurement can start is 2-3.5s after the start of the dissolution of the sample.

Microcontroller assembly. Fig. 1 also provides an overview over the microcontrollers (MC) used for the overall device. As micro-controller platforms we employed Arduino micro-controllers. The main criteria for selecting the Arduino platform is that it offers a built-in programming board attached to the micro-controller at a very low price and the Open Source implementation of the hardware and software. A MC controller design is superior over computer connected I/O interfaces.

We are using two Arduino Mega 2560 boards (based on the Atmel ATmega2560 micro-controller), clocked at 16 MHz frequency to control the valves and for the graphical display and an Arduino Nano V3 for the flow sensor. The first Arduino (MC1) is used to control the relay operations and timings of the valve assembly and the actuator of the multi-way valve. It receives a trigger from the optical liquid detector and triggers the spectrometer. MC2 is connected to a 3.2'' touch screen to enable user level I/O, and to set the trigger delay (600ms). The code for all 3 Arduino processors is provided as part of the supplementary material.

The Arduino Nano V3 (MC3) controls the liquid detector. For this we compared different concepts, starting from the implementation proposed by Bowen et al. who essentially used a capacitive conductivity detector. An altered design eliminated the need for an Operational Amplifier (OpAMp), thus also eliminating the need to Fourier transform the resulting signal (Fig. S5). The disadvantage of this design is that the actual detector consists of copper tubes placed around the PTFE sample tube connected to the output of the sample loop and this setup is highly sensitive towards mechanical rearrangements. Even the cable connecting this detector to its microcontroller platform had a severe influence on the signal detection.

We therefore tested *optical detectors*, starting with a home-built design using a plexiglas cell attached to a LED light and a light dependent resistor. Although this design worked more reliably it required the sample to pass through a transparent cell, which is not suitable for organic solvents. We finally selected a small commercially available detector (Optek Electronics OPB350) designed for 1/8'' PTFE tubes. We programmed an Arduino (MC3) (Fig. S4) to control the Optek sensor, which detects liquid entering the sensor and triggers MC1 to inject the sample. This setup works reliably and is very insensitive to movements of the tube or the selection of the sample or solvent. It also works with transparent samples such as pure water, does not require the colored radical in the solution and can be placed adjacent to the multi-way valve to minimize the dead volume.

MC1 and MC3 are interconnected through a serial interface. When serial commands are sent from the touch screen interface (Fig. S2), a program processes the inputs and produces the relevant outputs for the valves. An automatic event loop controls the timing of the overall process during the actual dissolution.

The design of the *sample holder* appeared to be a crucial bottleneck as it bears a significant risk to break NMR tubes under the high pressure p_{HP}. Our design (Fig. S6) was modified from Senczenko and Köckenberger [3]. The published design fitted a PEEK barrel tightly around the NMR tube by heating the PEEK until it softened. The disadvantage of this design is the abrupt change of pressure to the outside of the NMR tube where PEEK barrel ends causing regular breakage of NMR tubes at this position. To circumvent this we lined a barrel with a slightly larger diameter than the NMR tube with a thin layer of silicon. The softness of the silicon prevented NMR tubes from breaking but limits the maximum pressure to 15 bar. For safety reasons we used a medium walled NMR tube (Norell S-5-500-MW-7) with inner diameter of 3.43mm.

3. Experimental

NMR spectra. One-dimensional (1D) ^{13}C spectra were recorded with one scan without proton decoupling, a flip angle of 15° (8.12μs for 90° pulse) and 16k data points, 250ppm spectral width. Two-dimensional spectra were acquired using a small flip angle HMBC experiment as described earlier with 8k data points in the direct dimension and 16 increments, a 15° flip angle (90º pulse length of 8.12 μsec), a sweep width of 250ppm.
Longitudinal relaxation measurements were carried out as described by Day et al [4], using the single-scan FT (SSFT) method originally proposed by Kaptein [5]. A train of 30º pulses was employed, spaced 2sec apart with data acquisition during the first 500ms of this period with a spectral width of 250ppm. As described by Day a pulsed field gradient of 2 ms was employed to dephase remaining polarization.

DNP polarizations were carried out using an Oxford Instruments Hypersense polarizer (Oxford Instruments Molecular Biotools Ltd, Eynsham, UK) with typical polarization times for ^{13}C of 2-4h at 1.4K, typically using a 2mM concentration of the Ox63 radical [1]. For 1D experiments we polarized 100mM [U-^{13}C]glucose in 100μL of a D_2O:d_6-DMSO mixture (1:1), which forms a glass state after freezing. For 2D experiments we used 2M [U-^{13}C]glucose in the same solvent. For the dissolution we used 4 ml of a water:methanol (80:20) mixture. Samples were polarized for 2 hours for 2D spectra, including SSFT spectra, and 4 hours for 1D.

The pressures and timings of the dissolution device were optimized for transfer speed and sample stability. Considering that the pressure difference drives the sample transfer a larger difference between p_{HP} and p_{LP} is preferable, although p_{LP} must be sufficiently large to minimize forces on the NMR tube arising from the pressure jump. Additionally, the final pressure should be >10bar, the pressure used for the dissolution in the Hypersense. An optimal setup for our system was found for pressures of p_{HP}=12 and p_{LP}=3 bars, and a time delay of 600ms. Pressures and delay will depend on the individual implementation, in particular the distance from the polariser to the magnet (in our case les than 1.5m).

4. Results and Discussion

The optimized dissolution device was tested for sample stability. For this a series of pictures taken of the NMR tube as sample is transferred shows steady filling within 1.3s without forming bubbles arising from the gas in the sample (Fig. S7). This can be achieved reproducibly and without any sample breakage.

To test the device we polarised ^{13}C-labelled glucose for which it has been challenging to obtain NMR spectra owing to the fast relaxation of the glucose ^{13}C. Although the relaxation time of glucose can be increased by 30% using deuteration, which has enabled *in vivo* chemical shift imaging, deuteration removes the option to acquire ^{13}C-^{1}H-HMQC or spectra. Here we show that [6]we can obtain one-dimensional spectra reproducibly with a significantly improved quality. In dissolution DNP the sample suffers from a large load of gas dissolved in the sample owing to the high-pressure dissolution, which is typically carried out at 9-10bar. After this pressure is reduced the gas dissolved in the solvent is released causing bubbles. This results in low quality spectra owing to loss of homogeneity. For glucose with ^{13}C T_1 values of <1s a short transfer time is also essential. Fig. 2 shows the improvement achieved from the pressure dissolution device for a one-dimensional spectrum of [U-^{13}C]glucose with a transfer time of ~2s. While the spectrum shown in Fig. 1A suffers from bad resolution and low intensity (owing to a transfer time of ca 5s), the spectrum in panel B obtained with the dissolution device has excellent intensity and shows sharp lines, revealing the proton-carbon and carbon-carbon couplings.

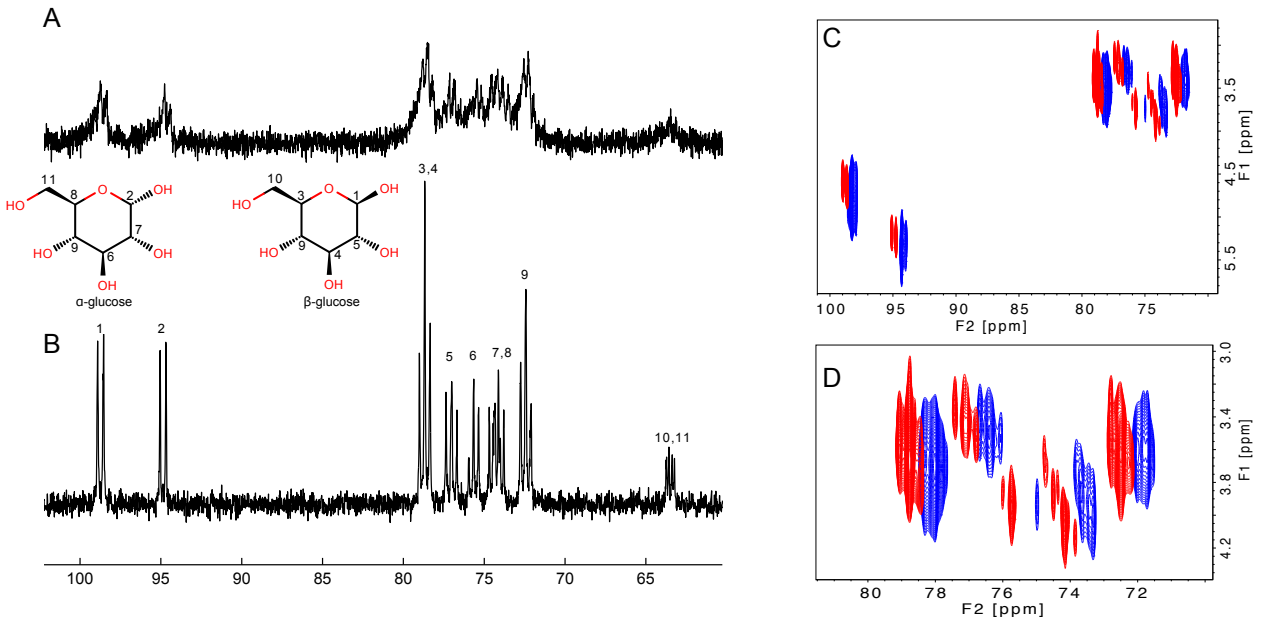

Fig. 2. A/B: 1D-^{13}C-spectrum of [U-^{13}C]glucose after 4h of polarization recorded (A) without, (B) with the pressure dissolution system. C/D: 2D small flip angle HMQC acquired after 2h of polarization without (blue) and with (red) the pressure dissolution system (the spectrum without the dissolution system has been slightly shifted for better visibility of the changes).

The pressure dissolution system has similar advantages for two-dimensional small flip angle HMQC spectra [7] as shown in Fig. 2C,D. Spectra obtained with the dissolution device shown I red have considerable lower line widths in the direct dimension and thus resolve details of the peak shapes. Considering the faster transfer it was also possible to acquire additional increments, thus improving the resolution in the indirect dimension.

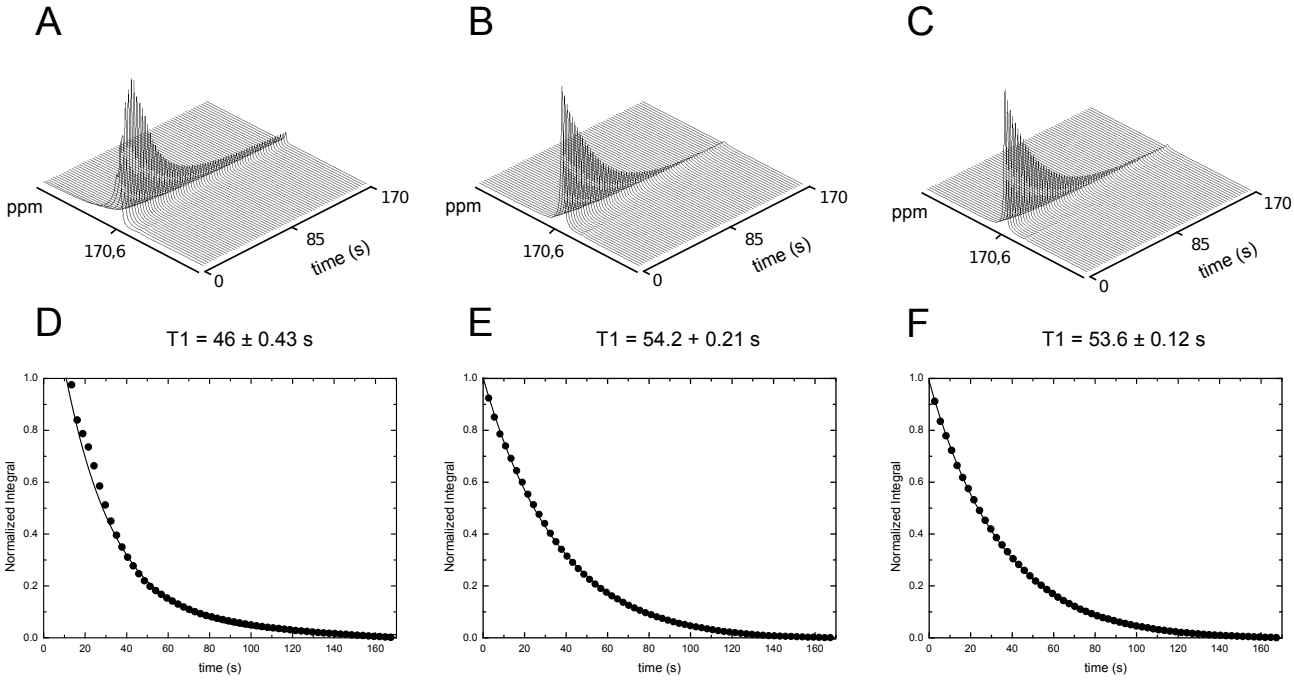

Fig. 3. A: Repeated sequential small flip angle spectra of hyperpolarized 1-^{13}C-pyruvate collected every 2s, acquired after a flip angle of 30° using the Hypersense transfer mechanism. B and C, same train of spectra using the pressure dissolution kit. D-F: SSFT data from A-C fitted using equation 8 from [4] to calculate a T_1 relaxation time.

In order to demonstrate the increased stability achieved with we carried out SSTF longitudinal relaxation time measurements using Day's method [4] (Fig. 3). Fig. 3A shows the expected instability arising from sample stabilization. For the simulation in Fig.3E the first 4 data points had to be omitted, as they show a build-up of signal arising from a slow sample stabilization over 8-10sec. Fig. 3B,c and F,G demonstrate the advantage obtain with the pressure dissolution kit. The T1 obtained was the same for two consecutive polarizations within small error limits. Data points could be used from time zero which is 600ms after the sample transfer was started.

In conclusion, we present a new design for a high-pressure dissolution device for dissolution DNP, which can be used along the Hypersense or any other implementations of this form of DNP. As part of our supplementary material we provide sufficient detail of the design to enable its implementation on other laboratories.

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